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Triacylglycerol Composition Profiling and Comparison of High-Oleic and Normal Peanut Oils

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Abstract Oilseed plants produce huge amounts of fatty acids (FA) stored as triacylglycerols (TAG) in seeds that give a great variation in their composition. The variety and content of TAG directly affect the nutrition and function of lipids. TAG composition of 12 high-oleic and normal peanut oil samples were profiled by two-dimensional liquid chromatography (2D LC) coupled with atmospheric pressure chemical ionization mass spectrometry (APCI-MS). The statistical evaluation of the TAG profiles determined was conducted on the basis of multidimensional data matrix using Principal Component Analysis (PCA). The technique enabled the differentiation of high-oleic oils from normal peanut oils—as results illustrated TAG of high-oleic peanut oil were clearly different from those of normal peanut oils. High-oleic and normal peanut oils had different profiles mainly in the contents of OOO, OPO and POL. This finding provided theoretical foundation for detecting the adulteration of edible oils and analyzing the nutrition and function of high-oleic peanut oils.

Keywords Triacylglycerols · High-oleic peanut oil · Principal component analysis

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Introduction

Peanuts (*Arachis hypogaea* L.), as one of the most important oil crops in China, contain approximately 50–55 % oil [1]. Oleic and linoleic acids constitute about 80 % of the fatty acid composition in peanut oil. More evidence showed that increasing the ratio of oleic to linoleic acid would improve the keeping quality of peanut oil. Therefore, improvement the stability of peanuts oil by modification of the oil composition has been the focus of peanut oil research [2, 3]. Unsaturated fatty acids of normal peanuts are mainly composed of 45 % oleic and 35 % linoleic acids. In comparison, high-oleic peanut oils contain approximately 80 % oleic and 2–3 % linoleic acid and have exhibited better properties than normal peanut oils. The anti-oxidation, chemical stability and sensory properties of high-oleic peanut oils are improved significantly throughout storage [4–9]. It has been reported that high-oleic peanut cultivars could be used to replace normal peanut cultivars without affecting consumer acceptance of peanut products despite minor differences in the volatile profile among the different genotypes peanut samples [10]. Furthermore, a diet high in oleic acid, which can be easily achieved through consumption of peanuts oil, has a beneficial effect on type II diabetes for reversing the negative effects of inflammatory cytokines observed in obesity and non-insulin dependent diabetes mellitus [11]. In addition, high-oleic peanuts have a potential role in preventing cardiovascular disease by reducing plasma low density lipoprotein-cholesterol (LDL) levels and raising high density lipoprotein-cholesterol (HDL) levels [12, 13]. Numerous research has concluded that improving the content of oleic fatty acid had no effect on peanut allergenicity and that high-oleic peanuts could not increase or decrease the risk of allergy [14]. Therefore, high-oleic peanut oils have attracted more research attention in recent years, and a growing line

of studies has revealed that the positive biological effects of high oleic peanut oils were mostly connected with its high oleic acid content [15].

The main constituents of peanut oils are TAG, which are esters composed primarily of three medium or long-chain fatty acids (FA) linked to a glycerol molecule. The characteristic of plant oils depending on their composition and comprehensive triacylglycerol (TAG) profiling can bring valuable information on their functions [16]. The distribution of fatty acids in triacylglycerol is not random, and different stereochemical positions of FA, namely *sn*-1, 2 or 3 on the glycerol backbone (regioisomers), lead to enormous complexity of the TAG structure. However, blending different TAG in the right proportion could lead to similar FA profiles. Consequently, the determination of the fatty acid composition is not sufficient to properly characterize a fat or an oil composition [17]. Moreover, due to the importance of TAG structure analysis for nutritional functions, quality control, technological characteristics and authenticity establishment, the physicochemical and nutritional properties of the oils have been determined by the TAG molecules. Therefore, recent studies tend to establish TAG composition as compositional markers in order to characterize fats and oils [18]. The types of TAG in high-oleic peanut oils are considered to be good fingerprints for quality and authenticity control, as well as for the nutritional value of the oil [19, 20]. In addition, the TAG composition of normal peanut oil has been reported [17, 21, 22], but TAG of high-oleic peanut oil have not been conducted. Traditionally, TAG of oils are primarily analyzed by high performance liquid chromatography (HPLC) and liquid chromatography mass spectrometry (LC–MS). Specially, non-aqueous reverse-phase HPLC (NARP-HPLC) and silver-ion HPLC (Ag-HPLC) are common employed for TAG separation [23–27]. However, the long analysis time and lower efficiency of those methods for dealing with large numbers of similar samples require developing an alternative method to fulfill the challenging work. Recently, our group proposed the application of two-dimensional liquid chromatography (2D LC) using a single column packed with silver-ion-modified octyl and sulfonic co-bonded silica coupled with atmospheric pressure chemical ionization - mass spectrometry (APCI-MS) for online profiling of TAG in plant oils. This novel 2D LC column combined the features of C8 column and silver-ion column, and exhibited much higher selectivity for the separation of TAG [28].

The objectives of this work are to analyze TAG of high-oleic and normal peanut oils in an attempt to characterize the various species of TAG of high-oleic peanut oil and evaluate the differences of TAG between the high-oleic and normal peanut oils. This research work will provide advanced information on the determination of TAG which are significant for nutrition and authenticity establishment of high-oleic peanut oils.

Materials and Methods

Abbreviation

The following abbreviations were used to indicate the FA bound to the glycerol backbone: M, myristic acid(C14:0); P, palmitic acid (C16:0); S, stearic acid (C18:0); O, oleic acid (C18:1, ⊿9); L, linoleic acid (C18:2, ⊿9, 12); Ln, linolenic acid (C18:3, ⊿9, 12, 15); A, arachidic acid (C20:0); G, gadoleic acid (C20:1, ⊿9); B, behenic acid (C22:0); Li, lignoceric acid (C24:0).

Materials and Reagents

Twelve cultivars and two experimental genotypes of raw, shelled peanut kernels were obtained from the Oil Crops Research Institute, Chinese Academy of Agricultural Sciences (CAAS), including the high-oleic peanuts cultivar "H-4107", "H-4108", "H-4109", "H-4110", "H-6101" and "H-6106" and the normal peanuts cultivar "N-3101", "N-3105", "N-3107", "N-3109","N-6107" and"N-6108". All of the peanut cultivars were grown, harvested and cured using conventional methods in China. Peanuts were shelled and passed through a 0.635 × 1.905 cm shaker screen, and then stored in plastic bags at 4 °C.

HPLC-grade hexane, 2-propanol, and ammonium hydroxide (NH_4OH) solution (10 %) were purchased from CNW (Düsseldorf, Germany), and HPLC-grade ACN was purchased from Merck (Darmstadt, Germany). Methanol and potassium hydroxide (KOH) were of analytical grade and obtained from Shanghai Chemical Co. (Shanghai, China). Clay (montmorillonite K 10, activity degree ≥200 mmol/kg, decolorization ratio ≥90 %) was purchased from Hangzhou Gangjin Chemical Co., Ltd (Hangzhou, China).

Preparation of Peanut Oils

Full, whole shelled peanuts with skins were used for extraction of oil by cold pressing using a press and centrifugation. The oil was then refined by decolorization (70 °C, 2.0 % activated clay, 10 min), degumming (70 °C, 2.0 % degumming clay, 10 min) and deacidification (70 °C, 3.0 % alkaline clay, 95 min). After centrifuging, the refined oil was purged with nitrogen, sealed in a glass bottle and then stored at 4 °C in a refrigerator.

Fatty Acids Analysis by Gas Chromatography

Fatty acid methyl esters (FAME) were prepared from the TAG in peanut oil using a standard procedure with a KOH–methanol solution (0.4 M) [29]. Analyses were carried out using an Agilent 7890 GC instrument (Agilent Technologies, Santa Clara, CA, USA) equipped with a FID and a

capillary column (FFAP, 30 m, 0.25 mm i.d., 0.5 μm film thickness). The GC conditions were as follows: carrier gas: nitrogen at an inlet pressure of 25 psi; injection volume: 1 μL; split ratio: 1:30; linear flow velocity: 1.5 mL/min; temperature program: initial temperature 210 °C, hold for 8 min, then ramp to 230 °C at 20 °C/min and hold for 8 min, with a total analysis time of 17 min. The temperatures of the injection port and detector were maintained at 250 and 280 °C, respectively. The fatty acid methyl esters were quantified using their relative peak area identified in the samples.

Liquid Chromatographic and MS Conditions

The instrument used was an Agilent 1200 series HPLC system equipped with a binary pump (model G1312A), a degasser (model G1379B), a autosampler (model G1329A) and a thermostatically controlled column compartment (model G1316A), all from Agilent Corporation, Palo Alto, CA, USA.

The silver modified HiSep OTS 2D column was prepared via ion exchange interactions between silver ions and sulfonic acid groups of the HiSep OTS column, according to a previously reported method [28].

The HPLC conditions were as below: column, silver-modified HiSep OTS 2D column; solvent, solvent A and solvent B (6:94, v/v), in which solvent A is H2O, and solvent B is methanol–acetonitrile (99:1, v/v); isocratic mode; flow rate, 1.0 mL/min; injection volume, 5 μL, oven temperature, 35 °C.

MS Conditions

The instrument used for MS analysis was a hybrid, triple quadrupole/linear IT mass spectrometer, API 4000 Q-Trap with an APCI interface (AB SCIEX, Foster City, CA, USA). The conditions were as below: APCI mode: positive; CUR (curtain gas) pressure: 137.9 kPa; CAD (collision gas): medium; NC (nebulizer current): 27.58 kPa; TEM (temperature): 450 °C; scan mode: EMS (enhanced product ion), or MRM (multiple reaction monitoring); scan rate: 250 scan/s; GS1 (ion source gas 1) pressure: 344.75 kPa; interface heater: on; DP (declustering potential): 90 V; CE (collision energy): 45 V; collision energy spread: 5 V; CXP (collision cell exit potential): 17 V; mass range: 500-1,000 m/z. The EMS and MRM mode was applied for qualitative analysis and quantification of TAG, respectively. Selected reaction monitoring conditions for protonated TAG are listed in Table 1.

Identification and Quantification Analysis of Peanut Oils

TAG in peanut oils were identified by IntelliXtract software from Advanced Chemistry Development, Inc. (USA) on the basis of their positive-ion APCI mass spectra. The $[M + H]^+$ ions and $[M + H\text{-}RiCOOH]^+$ fragment ions were used for the weight determination and the identification of individual FA in EMS scan mode, respectively. A total of 58 kinds of TAG in high-oleic and normal peanut oils were identified in this study.

Trimyristin (MMM), which was not contained in the 12 peanut oil samples, was used as an IS to quantify TAG in oil samples. The relative peak areas (analyte area/IS area) were used for quantification of the TAG. Triplicate measurements were performed in MRM scan mode, and average values were used for analysis.

Multivariate Statistical Analysis

For current study, the 12 peanut oil samples were designated as objects (rows), and the relative peak areas of the 58 identified TAG were variables (columns). The data set for multivariate statistical analysis was processed using MetaboAnalyst 2.0 (http://www.metaboanalyst.ca/MetaboAnalyst/) without any additional pretreatment. 2D PCA score plots and loading plot were created from the data, with PC1 being the axis that contained the largest possible amount of information and PC2 being the axis perpendicular to PC1. The principal components were the orthogonal and linear combinations of the original variables. PCA score plots were used to model the relationship of TAG compounds in oils obtained from different plants. 12 samples of peanut oils were conducted in triplicate.

Results and Discussion

Fatty Acids Analysis

The fatty acid compositions of high-oleic and normal peanuts oils were shown in Table 2. There were significant differences ($p < 0.01$) between the content of oleic acid and linoleic acid in the high-oleic oils and the normal peanuts oil as linoleic acid was replaced by oleic acid in the high-oleic peanut oils. From Table 2, the content of oleic acid and - linoleic acid was in the range of 76.31–80.08 % and 1.70–3.56 % for the high oleic peanut oils, respectively. These results corresponded with those from other studies and showed that the sum of oleic and linoleic acids accounted for almost 80 % of the total fatty acids detected in peanut oil samples [30, 31]. On the other hand, the content of oleic acid in normal oleic oils was from 39.48 to 46.79 % while the content of linoleic acid was 30.12–37.60 %. Comparing the two types of oils, there was strong statistical significant ($p < 0.01$) differences in palmitic acid, gadoleic acid and lignoceric acid in addition to oleic acid and linoleic

Table 1 Retention times (min), $[M + H]^+$ observed, formulae, fragments, TAG and ECN of the TAG identified in high-oleic and normal peanut oils

Peak no.	Rt (min)	$[M + H]^+$observed	Formulae	Fragments	TAG[a]	ECN
1	11.72	879.8	$C_{57}H_{98}O_6$	597.6,599.6,601.6	OLLn[b]	42
1	11.72	879.8	$C_{57}H_{98}O_6$	599.6	LLL	42
2	12.45	855.8	$C_{55}H_{98}O_6$	599.9,577.6,573.6	PLnO[c]	44
2	12.45	855.8	$C_{55}H_{98}O_6$	599.6,575.6	LLP	44
3	12.90	881.8	$C_{57}H_{100}O_6$	599.6,601.6	OLL[b]	44
3	12.90	881.8	$C_{57}H_{100}O_6$	599.6,603.6	OOLn	44
4	13.85	857.8	$C_{55}H_{100}O_6$	601.6,577.6,575.6	POL[d]	46
5	14.22	883.8	$C_{57}H_{102}O_6$	599.6,601.6,605.6	SOLn[b]	46
5	14.22	883.8	$C_{57}H_{102}O_6$	601.6,603.6	OLO	46
6	14.78	833.8	$C_{53}H_{100}O_6$	577.6,551.6	POP[d]	48
7	15.34	859.8	$C_{55}H_{102}O_6$	577.6,603.6	OPO[b]	48
7	15.34	859.8	$C_{55}H_{102}O_6$	579.6,575.6,603.6	SPL	48
8	15.65	885.8	$C_{57}H_{104}O_6$	629.6,605.6,575.6	PLGb	48
8	15.65	885.8	$C_{57}H_{104}O_6$	601.6,603.6,605.6	SOL[b]	48
8	15.65	885.8	$C_{57}H_{104}O_6$	603.6	OOO	48
9	16.40	911.8	$C_{59}H_{106}O_6$	631.6,599.6	LLA[c]	48
9	16.40	911.8	$C_{59}H_{106}O_6$	633.6,629.6,599.6	LnOA	48
9	16.40	911.8	$C_{59}H_{106}O_6$	629.6,631.6,601.6	OLG	48
10	17.22	861.8	$C_{55}H_{104}O_6$	605.6,579.6,577.6	PSOc	50
11	17.91	887.8	$C_{57}H_{106}O_6$	603.6,607.6	SSL[c]	50
11	17.91	887.8	$C_{57}H_{106}O_6$	631.6,575.6,607.6	PAL	50
11	17.91	887.8	$C_{57}H_{106}O_6$	631.6,577.6,605.6	POG[b]	50
11	17.91	887.8	$C_{57}H_{106}O_6$	603.6,605.6	OSO	50
12	18.43	913.8	$C_{59}H_{108}O_6$	633.6,631.6,601.6	LOA[b]	50
12	18.43	913.8	$C_{59}H_{108}O_6$	631.6,603.6	OGO	50
13	19.17	939.8	$C_{61}H_{110}O_6$	661.6,657.6,599.6	LnOB[c]	50
13	19.17	939.8	$C_{61}H_{110}O_6$	659.6,599.6	LLB	50
14	20.48	889.8	$C_{57}H_{108}O_6$	605.6,607.6	SSO[b]	52
14	20.48	889.8	$C_{57}H_{108}O_6$	633.6,607.6,577.6	APO[d]	52
15	21.00	915.8	$C_{59}H_{110}O_6$	659.6,575.6,635.6	PBL[b]	52
15	21.00	915.8	$C_{59}H_{110}O_6$	635.6,631.6,603.6	LSA	52
15	21.00	915.8	$C_{59}H_{110}O_6$	633.6,603.6	OAO[d]	52
16	22.09	941.8	$C_{61}H_{112}O_6$	659.6,661.6,601.6	OLB[d]	52
17	22.65	967.8	$C_{63}H_{114}O_6$	599.6,689.6,687.6	OLnLi[d]	52
18	23.92	917.8	$C_{59}H_{112}O_6$	633.6,635.6,605.6	SAO[b]	54
18	23.92	917.8	$C_{59}H_{112}O_6$	661.6,635.6,577.6	POB[d]	54
19	25.05	943.8	$C_{61}H_{114}O_6$	663.6,659.6,603.6	LSB	54
19	25.05	943.8	$C_{61}H_{114}O_6$	661.6,603.6	OBO	54
19	25.05	943.8	$C_{61}H_{114}O_6$	687.6,663.6,575.6	PLiL	54
20	26.02	969.8	$C_{63}H_{116}O_6$	659.6,689.6,629.6	GLB[c]	54
20	26.02	969.8	$C_{63}H_{116}O_6$	687.6,689.6,601.6	OLLi[d]	54
21	28.48	945.8	$C_{61}H_{116}O_6$	689.6,663.6,577.6	POLi[b]	56
21	28.48	945.8	$C_{61}H_{116}O_6$	661.6,663.6,605.6	SOB	56

Rt Retention time

[a] Structure is indicated by fatty acid composition: *P* palmitic acid (C16:0), *S* stearic acid (C18:0), *O* oleic acid (C18:1, ⊿9), *L* linoleic acid (C18:2, ⊿9, 12), *Ln* linolenic acid (C18:3, ⊿9, 12, 15), *A* arachidic acid (C20:0), *G* gadoleic acid (C20:1, ⊿9), *B* behenic acid (C22:0), *Li* lignoceric acid (C24:0)

[b] Co-eluted with the next TAG, and the regioisomers could not be identified

[c] Co-eluted with the next TAG, but the regioisomers could be identified

[d] Regioisomers could not be identified

acid. Statistical significant differences ($p < 0.05$) were also observed in the content of behenic acid. There were no differences in the content of stearic acid. The total saturated fatty acids were significantly ($p < 0.01$) lower in high-oleic oil than the normal peanut oils. This was caused by the lower palmitic levels in the high-oleic oils, as the content of other saturated fatty acids were not far different in both oils.

Table 2 Fatty acids composition of high-oleic and normal peanut oil (relative content, %, w/w)

Fatty acids	N-3101	N-3105	N-3107	N-3109	N-6107	N-6108	H-4107	H-4108	H-4109	H-4110	H-6101	H-6106	Significance[a]
Palmitic acid (C16:0)	11.48 ± 0.28	12.73 ± 0.26	11.26 ± 0.15	13.36 ± 0.21	11.26 ± 0.32	11.10 ± 0.21	6.56 ± 0.24	6.48 ± 0.18	6.72 ± 0.22	6.73 ± 0.19	6.50 ± 0.25	6.19 ± 0.20	**
Stearic acid (C18:0)	5.36 ± 0.12	3.83 ± 0.15	4.89 ± 0.13	4.17 ± 0.16	4.36 ± 0.12	4.92 ± 0.21	6.08 ± 0.13	4.29 ± 0.15	4.79 ± 0.24	3.38 ± 0.14	5.16 ± 0.27	4.25 ± 0.13	N
Oleic acid (C18:1)	46.24 ± 0.43	39.48 ± 0.59	44.99 ± 0.61	41.75 ± 0.58	46.79 ± 0.44	43.50 ± 0.55	77.27 ± 0.75	78.43 ± 0.65	76.31 ± 0.87	80.08 ± 0.76	78.43 ± 0.80	78.65 ± 0.75	**
Linoleic acid (C18:2)	30.12 ± 0.36	37.60 ± 0.48	31.87 ± 0.54	33.77 ± 0.49	30.19 ± 0.55	33.28 ± 0.61	1.70 ± 0.05	2.21 ± 0.10	3.56 ± 0.11	2.25 ± 0.11	1.47 ± 0.08	2.35 ± 0.11	**
Linolenic acid (C18:3)	0.47 ± 0.03	0.24 ± 0.02	0.42 ± 0.02	0.29 ± 0.01	0.53 ± 0.03	0.26 ± 0.02	0.42 ± 0.02	0.57 ± 0.03	0.62 ± 0.02	0.61 ± 0.04	0.69 ± 0.03	0.58 ± 0.02	**
Arachidic acid (C20:0)	1.96 ± 0.06	1.56 ± 0.03	1.96 ± 0.01	1.71 ± 0.02	1.87 ± 0.03	2.06 ± 0.01	2.23 ± 0.03	1.88 ± 0.02	2.03 ± 0.04	1.51 ± 0.03	2.08 ± 0.03	1.93 ± 0.02	N
Gadoleic acid (C20:1)	0.60 ± 0.03	0.64 ± 0.02	0.64 ± 0.03	0.70 ± 0.02	0.75 ± 0.01	0.66 ± 0.02	0.99 ± 0.03	1.15 ± 0.03	1.12 ± 0.04	1.44 ± 0.03	1.19 ± 0.04	1.31 ± 0.03	**
Behenic acid (C22:0)	2.43 ± 0.07	2.43 ± 0.10	2.71 ± 0.09	2.81 ± 0.09	2.77 ± 0.08	2.79 ± 0.07	3.22 ± 0.09	3.37 ± 0.09	3.24 ± 0.10	2.55 ± 0.09	2.94 ± 0.09	3.12 ± 0.10	*
Lignoceric acid (C24:0)	1.35 ± 0.03	1.51 ± 0.05	1.27 ± 0.04	1.43 ± 0.05	1.47 ± 0.06	1.43 ± 0.03	1.52 ± 0.04	1.65 ± 0.05	1.65 ± 0.04	1.46 ± 0.03	1.54 ± 0.04	1.63 ± 0.06	**
Saturated fatty acids	22.57	22.04	22.08	23.49	21.74	22.3	19.62	17.64	18.39	15.62	18.22	17.11	**
Unsaturated fatty acids	77.43	77.96	77.92	76.51	78.26	77.7	80.38	82.36	81.61	84.38	81.78	82.89	**

The content of fatty acids was the mean ± SD ($n = 3$). The data were analyzed using analysis of variance (ANOVA)

N no difference

* $P < 0.05$

** $P < 0.01$

[a] The differences between high-oleic and normal peanut oil group

Table 3 Quantitative results (relative content, %, w/w) of TAG from high-oleic and normal peanuts oils

TAG[a]	Normal peanut oils						High-oleic peanut oils					
	N-3101	N-3105	N-3107	N-3109	N-6107	N-6108	H-4107	H-4108	H-4109	H-4110	H-6101	H-6106
OLLn[b]	0.99 ± 0.05	2.09 ± 0.09	1.24 ± 0.08	1.49 ± 0.10	1.35 ± 0.07	1.21 ± 0.08	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.02 ± 0.00
LLL	2.76 ± 0.11	5.18 ± 0.23	2.88 ± 0.10	3.22 ± 0.13	2.80 ± 0.11	2.98 ± 0.12	0.01 ± 0.00	0.01 ± 0.00	0.06 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
OLL[b]	6.55 ± 0.18	7.79 ± 0.17	7.73 ± 0.15	9.27 ± 0.21	6.78 ± 0.17	7.30 ± 0.19	0.05 ± 0.00	0.08 ± 0.00	0.29 ± 0.02	0.07 ± 0.00	0.03 ± 0.00	0.15 ± 0.01
OOLn	2.64 ± 0.12	3.11 ± 0.11	3.01 ± 0.13	3.54 ± 0.12	2.70 ± 0.14	2.80 ± 0.11	0.03 ± 0.00	0.04 ± 0.00	0.13 ± 0.01	0.05 ± 0.00	0.03 ± 0.00	0.09 ± 0.00
PLnO[c]	1.39 ± 0.09	1.74 ± 0.11	1.21 ± 0.08	1.81 ± 0.09	1.50 ± 0.08	1.36 ± 0.09	0.02 ± 0.00	0.02 ± 0.00	0.06 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.03 ± 0.00
LLP	2.97 ± 0.11	4.63 ± 0.18	3.59 ± 0.15	3.95 ± 0.17	2.75 ± 0.16	3.58 ± 0.20	0.03 ± 0.00	0.04 ± 0.00	0.10 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.07 ± 0.00
POL[d]	12.02 ± 0.35	15.10 ± 0.40	12.52 ± 0.38	15.81 ± 0.32	12.73 ± 0.27	12.47 ± 0.31	0.50 ± 0.03	0.59 ± 0.05	1.04 ± 0.07	0.67 ± 0.04	0.50 ± 0.05	0.76 ± 0.07
SOLn[b]	2.96 ± 0.15	3.02 ± 0.12	2.76 ± 0.14	2.73 ± 0.15	2.57 ± 0.19	3.27 ± 0.20	0.05 ± 0.00	0.08 ± 0.01	0.16 ± 0.01	0.06 ± 0.00	0.05 ± 0.00	0.11 ± 0.01
OLO	6.72 ± 0.19	7.05 ± 0.18	7.06 ± 0.21	7.22 ± 0.15	7.60 ± 0.22	6.65 ± 0.23	0.40 ± 0.01	0.65 ± 0.02	1.00 ± 0.05	0.73 ± 0.04	0.47 ± 0.05	0.79 ± 0.08
POP[d]	2.45 ± 0.13	2.39 ± 0.15	1.90 ± 0.16	2.74 ± 0.11	2.43 ± 0.18	2.37 ± 0.17	1.38 ± 0.09	1.25 ± 0.10	1.21 ± 0.11	0.95 ± 0.08	1.09 ± 0.12	1.19 ± 0.10
OPO[b]	8.71 ± 0.20	8.08 ± 0.17	8.22 ± 0.24	8.53 ± 0.21	8.72 ± 0.29	7.71 ± 0.21	9.85 ± 0.25	9.92 ± 0.24	9.26 ± 0.28	10.30 ± 0.30	10.12 ± 0.25	10.05 ± 0.21
SPL	0.99 ± 0.08	0.92 ± 0.07	0.77 ± 0.08	1.01 ± 0.09	0.78 ± 0.08	1.14 ± 0.09	0.10 ± 0.00	0.08 ± 0.00	0.10 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.08 ± 0.00
PLG[c]	0.34 ± 0.05	0.55 ± 0.06	0.38 ± 0.05	0.45 ± 0.07	0.40 ± 0.04	0.41 ± 0.06	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
SOL[b]	5.01 ± 0.15	4.24 ± 0.16	4.42 ± 0.14	4.05 ± 0.15	3.90 ± 0.17	4.90 ± 0.16	1.09 ± 0.09	0.99 ± 0.10	1.16 ± 0.12	0.96 ± 0.09	0.89 ± 0.09	1.05 ± 0.11
OOO	23.21 ± 0.45	15.62 ± 0.37	21.89 ± 0.42	16.68 ± 0.35	25.40 ± 0.43	22.19 ± 0.37	59.60 ± 0.43	62.93 ± 0.50	62.68 ± 0.46	67.81 ± 0.51	61.23 ± 0.53	61.90 ± 0.42
LLA[c]	0.83 ± 0.07	1.03 ± 0.08	0.95 ± 0.07	0.83 ± 0.06	0.45 ± 0.04	0.87 ± 0.06	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
LnOA	0.36 ± 0.04	0.47 ± 0.03	0.40 ± 0.03	0.38 ± 0.04	0.25 ± 0.04	0.48 ± 0.03	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
OLG	0.17 ± 0.02	0.23 ± 0.02	0.21 ± 0.02	0.29 ± 0.03	0.24 ± 0.02	0.22 ± 0.02	0.03 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
PSO[d]	0.83 ± 0.07	0.55 ± 0.06	0.66 ± 0.06	0.62 ± 0.05	0.68 ± 0.06	0.62 ± 0.05	0.76 ± 0.06	0.59 ± 0.05	0.60 ± 0.05	0.37 ± 0.03	0.58 ± 0.05	0.51 ± 0.04
SSL[c]	0.34 ± 0.03	0.36 ± 0.04	0.39 ± 0.03	0.29 ± 0.03	0.31 ± 0.02	0.46 ± 0.45	0.09 ± 0.01	0.06 ± 0.00	0.07 ± 0.00	0.03 ± 0.00	0.06 ± 0.00	0.07 ± 0.00
PAL	0.19 ± 0.02	0.22 ± 0.02	0.21 ± 0.02	0.19 ± 0.02	0.19 ± 0.01	0.20 ± 0.03	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
POG[b]	0.18 ± 0.02	0.19 ± 0.01	0.15 ± 0.01	0.25 ± 0.02	0.18 ± 0.01	0.18 ± 0.02	0.29 ± 0.02	0.31 ± 0.03	0.26 ± 0.02	0.35 ± 0.03	0.31 ± 0.02	0.35 ± 0.03
OSO	5.04 ± 0.29	2.67 ± 0.11	4.43 ± 0.23	2.76 ± 0.19	3.19 ± 0.21	4.00 ± 0.23	10.52 ± 0.31	7.30 ± 0.29	7.45 ± 0.35	5.97 ± 0.32	8.59 ± 0.37	7.74 ± 0.41
LOA[b]	1.69 ± 0.09	1.38 ± 0.11	1.58 ± 0.12	1.29 ± 0.11	1.37 ± 0.17	1.60 ± 0.12	0.13 ± 0.01	0.15 ± 0.01	0.21 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.19 ± 0.01
OGO	0.29 ± 0.02	0.24 ± 0.02	0.29 ± 0.02	0.25 ± 0.02	0.32 ± 0.02	0.26 ± 0.01	0.95 ± 0.05	1.05 ± 0.07	1.06 ± 0.06	1.40 ± 0.08	1.21 ± 0.09	1.38 ± 0.09
LnOB[c]	0.34 ± 0.02	0.53 ± 0.03	0.34 ± 0.02	0.44 ± 0.02	0.36 ± 0.02	0.47 ± 0.03	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.04 ± 0.00
LLB	0.40 ± 0.02	0.65 ± 0.03	0.46 ± 0.02	0.51 ± 0.03	0.43 ± 0.02	0.50 ± 0.03	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.04 ± 0.00
SOS[b]	0.43 ± 0.02	0.19 ± 0.01	0.31 ± 0.02	0.13 ± 0.01	0.29 ± 0.02	0.28 ± 0.02	0.84 ± 0.05	0.41 ± 0.03	0.52 ± 0.03	0.25 ± 0.02	0.59 ± 0.03	0.38 ± 0.02
APO[d]	0.42 ± 0.02	0.36 ± 0.02	0.42 ± 0.02	0.36 ± 0.01	0.37 ± 0.02	0.36 ± 0.02	0.50 ± 0.03	0.44 ± 0.02	0.40 ± 0.02	0.33 ± 0.02	0.38 ± 0.02	0.35 ± 0.02
PBL[b]	0.49 ± 0.03	0.64 ± 0.04	0.55 ± 0.05	0.57 ± 0.03	0.54 ± 0.04	0.58 ± 0.03	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.00	0.06 ± 0.00	0.07 ± 0.00
LSA	0.13 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.14 ± 0.02	0.11 ± 0.01	0.08 ± 0.00	0.10 ± 0.00	0.08 ± 0.01	0.12 ± 0.01	0.10 ± 0.00
OAO[d]	1.87 ± 0.11	1.26 ± 0.08	1.82 ± 0.12	1.19 ± 0.10	1.56 ± 0.12	1.61 ± 0.11	4.55 ± 0.25	3.68 ± 0.19	3.91 ± 0.20	3.43 ± 0.17	4.80 ± 0.21	4.15 ± 0.18
OLBc	1.37 ± 0.10	1.62 ± 0.12	1.72 ± 0.11	1.61 ± 0.13	1.51 ± 0.12	1.58 ± 0.13	0.24 ± 0.02	0.29 ± 0.01	0.37 ± 0.02	0.30 ± 0.02	0.29 ± 0.02	0.39 ± 0.03
OLnLi[d]	0.84 ± 0.04	1.31 ± 0.08	0.98 ± 0.05	1.05 ± 0.05	0.80 ± 0.04	0.91 ± 0.05	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.04 ± 0.00

Table 3 continued

TAG[a]	Normal peanut oils						High-oleic peanut oils					
	N-3101	N-3105	N-3107	N-3109	N-6107	N-6108	H-4107	H-4108	H-4109	H-4110	H-6101	H-6106
SAO[b]	0.14 ± 0.02	0.07 ± 0.01	0.13 ± 0.02	0.07 ± 0.01	0.11 ± 0.01	0.14 ± 0.02	0.33 ± 0.02	0.21 ± 0.02	0.24 ± 0.01	0.08 ± 0.01	0.25 ± 0.02	0.17 ± 0.01
POB[d]	0.43 ± 0.03	0.46 ± 0.04	0.49 ± 0.04	0.51 ± 0.03	0.48 ± 0.04	0.48 ± 0.03	0.66 ± 0.03	0.69 ± 0.05	0.58 ± 0.04	0.37 ± 0.03	0.53 ± 0.03	0.53 ± 0.04
LSB	0.10 ± 0.01	0.12 ± 0.01	0.11 ± 0.00	0.12 ± 0.01	0.11 ± 0.00	0.14 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
OBO	1.98 ± 0.11	1.79 ± 0.13	2.02 ± 0.19	1.85 ± 0.20	2.19 ± 0.18	1.84 ± 0.15	6.02 ± 0.28	7.17 ± 0.31	5.88 ± 0.29	4.18 ± 0.23	6.69 ± 0.35	6.33 ± 0.32
PLiL	0.21 ± 0.02	0.37 ± 0.02	0.23 ± 0.01	0.29 ± 0.02	0.25 ± 0.02	0.22 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
GLB[c]	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
OLLi[d]	1.00 ± 0.05	1.38 ± 0.06	1.18 ± 0.05	1.25 ± 0.06	1.04 ± 0.08	1.24 ± 0.07	0.11 ± 0.01	0.15 ± 0.01	0.21 ± 0.02	0.21 ± 0.02	0.17 ± 0.01	0.26 ± 0.02
POLi[b]	0.13 ± 0.01	0.22 ± 0.02	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.18 ± 0.02	0.23 ± 0.02	0.24 ± 0.02	0.24 ± 0.02	0.26 ± 0.02	0.25 ± 0.01	0.24 ± 0.02
SOB	0.08 ± 0.01	0.06 ± 0.00	0.09 ± 0.00	0.10 ± 0.01	0.08 ± 0.00	0.09 ± 0.00	0.26 ± 0.02	0.18 ± 0.01	0.21 ± 0.01	0.15 ± 0.01	0.21 ± 0.02	0.18 ± 0.01

[a] Structure is indicated by fatty acid composition: *P* palmitic acid (C16:0), *S* stearic acid (C18:0), *O* oleic acid (C18:1, $\Delta 9$), *L* linoleic acid (C18:2, $\Delta 9$, 12), *Ln* linolenic acid (C18:3, $\Delta 9$, 12, 15), *A* arachidic acid (C20:0), *G* gadoleic acid (C20:1, $\Delta 9$), *B* behenic acid (C22:0), *Li* lignoceric acid (C24:0)

[b] Co-eluted with the next TAG, and the regioisomers could not be identified

[c] Co-eluted with the next TAG, but the regioisomers could be identified

[d] Regioisomers could not be identified

Profiling of TAG from Peanut Oils

With the presence of numerous TAG species and double bond, the separation of TAG from plant oils has been a great challenging task. Non-aqueous reversed-phase HPLC (NARP-HPLC) separation mode was used for the separation of TAG complex mixtures of plant oils based on equivalent carbon number (ECN) of TAG. However, in Ag-HPLC, the TAG retention behavior was closely connected to the number and position of double bonds (DB) of TAG. In this study, TAG composition of peanut oils were profiled by 2D LC using a single column which combined the features of a C8 column and silver-ion, coupled with APCI-MS. Individual TAG were identified by APCI-MS on the basis of their positive-ion $[M + H]^+$ for the molecular weight determination and $[M + H\text{-RiCOOH}]^+$ fragment ions for the identification of corresponding fatty acids. Combining with 9 fatty acids identified above by GC, in total, 58 TAG species were identified in peanut oils composed of 16–24 carbon atoms and 0–3 DB.

The TAG profile for high-oleic and normal peanut oils were presented in Table 3, which revealed distinct differences in the composition of high-oleic and normal peanuts oils. The main TAG in normal peanut oils were OOO (15.62–25.40 %), POL (12.02–15.81 %), OPO (7.71–8.72 %), OLL (6.55–9.27 %) and OLO (6.65–7.60 %) while the predominant TAG in high-oleic peanut oils were OOO (59.60–67.81 %), OSO (5.97–10.52 %) and OBO (4.18–7.17 %) which were found only in trace amount in normal peanuts oils. The content of LLL, OLL, OOLn, POL and OLO in normal peanut oils was significantly higher than those in high-oleic peanut oils. The results were in good agreement with fatty acids analysis described in Table 2. The most abundant oleic acid-containing TAG species is found to be OOO with the next-most abundant being POL, OLL and OLO. When oleic acid concentration increased and linoleic acid concentration decreased, the percentage distribution of OOO raised. The relative content of oleic acid had a positive direct impact on OOO concentration in peanuts oil. Oleic acid was a precursor of OOO, and thus high relative content of oleic acid obtained high concentration OOO. Sanders [21, 22] described the variability existing in the stereospecific structure of triacylglycerols from six peanut varieties. The percentage of palmitic and stearic acids, generally very low at the *sn*-2 position, were more predominant at the *sn*-1 than the *sn*-3 position. The *sn*-2 position of all varieties was high in unsaturated fatty acids. Generally, a higher percentage of oleic acid in the triacylglycerol resulted in a greater proportion of OOO.

Principal Components Analysis of TAG Composition in High-Oleic and Normal Peanut Oils

A principal component analysis (PCA) was performed to simplify data from TAG profiles of peanuts oils. Figure 1

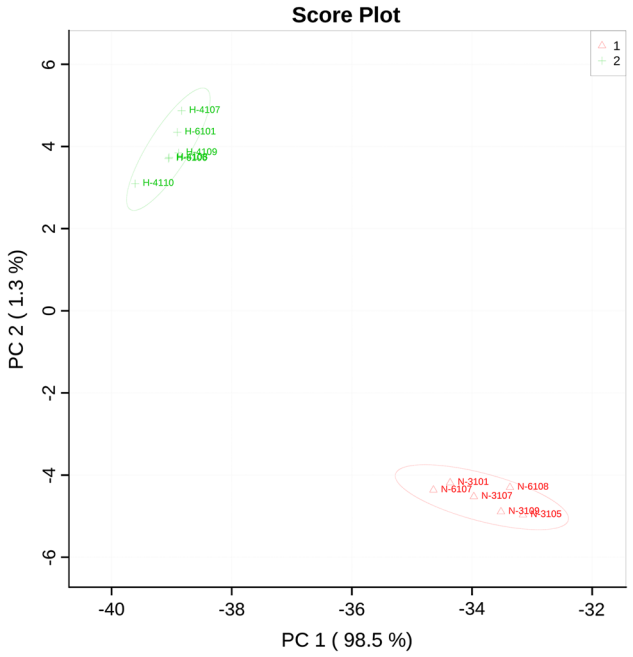

Fig. 1 Score plot of principal component analysis based on TAG compound profiling analysis of all samples: six high-oleic peanut oils (H-4107, H-4108, H-4109, H-4110, H-6101 and H-6106) and six normal peanut oils (N-3101, N-3105, N-3107, N-3109, N-6107 and N-6108)

showed the score plot of the first principal component (PC1) and second principal component (PC2) of all peanuts oil samples. This data set was represented by 12 objects (oil samples) and 43 variables (TAG concentrations) with significant variability. PC1 and PC2 accounted for 99.8 % of total variability, where PC1 represented 98.5 % and PC2 represented 1.3 % of total variability. The score plot of the PC1–PC2 comparison revealed two distinct groups of samples. On the top of the plots—i.e. for the values of PC2 > 0 all of normal peanut oil samples were located. And high-oleic peanut oils were scattered in the lower part of the planes. These results indicated that TAG composition from high-oleic peanut oils were different from that of normal peanut oil.

Figure 2 shows PCA loading plots in the plane of PC1 and PC2. Their variance model mostly affected the variability of samples. TAG with similar levels in each oil analysis gathered in the middle right part of the loading plot, whereas TAG with significantly different levels in each oil analysis scattered at the edges of the loading plot. The loading plot showed that for the first component (98.5 % explained variation) the most important variables were: OOO, and OPO. For the second component (1.3 % explained variation) the most important variable was POL

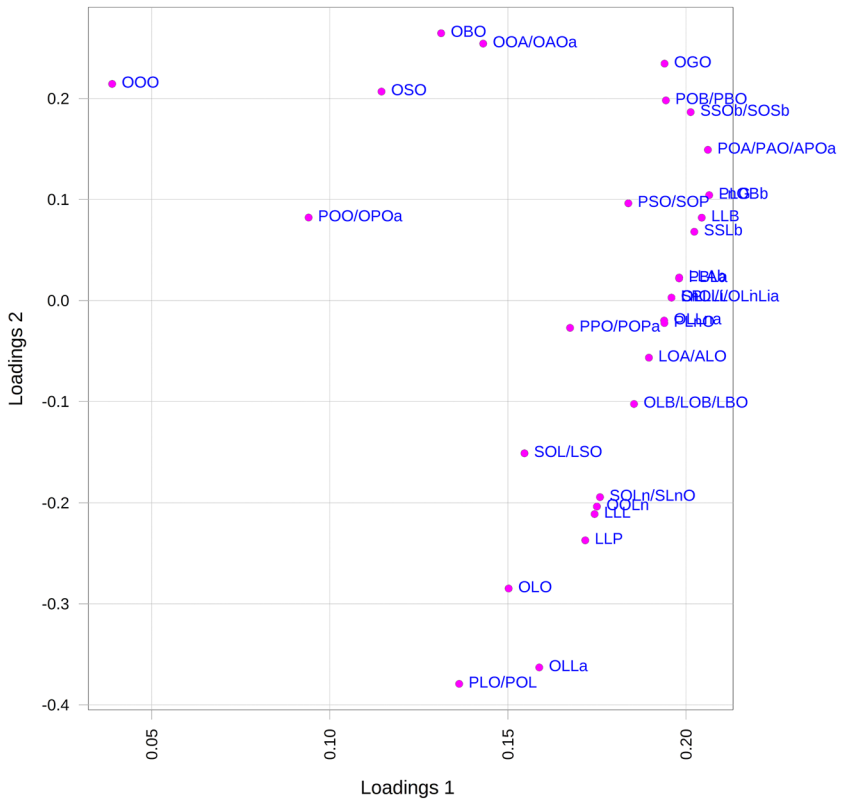

Fig. 2 Projection of variables in a two-dimensional loading plot for all measured samples, showing the major variables representing TAG concentrations

content. Thus the amounts of OOO, OPO and POL were the most significant variables for the statistical differentiation among high-oleic and normal peanut oil. As expected, high-oleic and normal peanuts oils could be easily differentiated from the levels of OOO. It was also clear from these results that this parameter could be a useful tool in the identification and discrimination of vegetable oils. Furthermore, it might be an important parameter to detect the adulteration of such products during quality control.

When considering the nutritional effects of edible oils, TAG structure and the species composition affected the digestion and absorption of TAG in addition to the overall fatty acid profile. The positional distribution of fatty acids in dietary TAG determined whether fatty acids were absorbed as 2-monoglycerides or free fatty acids [32]. In the process of digestion and absorption, the fatty acids in the *sn*-1 position and in the *sn*-3 position would be more easily hydrolyzed from the TAG structure than those in *sn*-2 position. In addition, some researchers found that saturated fatty acids from cocoa butter, which were located solely in the *sn*-1,3 positions, were lost in feces, whereas C18:1, which was located in the *sn*-2 position, was incorporated into the epididymal fat tissue [33]. Moreover, oleic acid was the most abundant fatty acid in human adipose tissue. Many of the health effects of olive oil were usually attributed to its high oleic acid content. Research suggested that Mediterranean diet which was rich in mono-unsaturated fats helped to prevent coronary artery disease and stroke because of its healthy lipid profile [34]. Therefore, profiling of TAG from high-oleic peanut oil was beneficial to nutrition research of peanut oil and provided valuable information for adulteration of edible oil.

Conclusions

This research showed the successful characterization of TAG of high-oleic and normal peanut oils using 2D LC coupled with APCI-MS and indicated the differentiation of individual TAG of the oils achieved by PCA. A clear resolution of high-oleic and normal peanut oil samples and their grouping into small clusters enable the resolution of model samples of adulterated high-oleic peanut oil by normal peanut oil. High-oleic and normal peanut oils had different profiles mainly in the contents of OOO, OPO and POL. Furthermore, this study clearly indicated that the combination of experimental TAG data along with a chemometric approach (PCA, in this case) could be successfully employed by researchers in collaboration with the peanut industry to give more information on the nutrition aspect of the oils.

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